The Flavor and Fragrance High Production Volume Consortia (FFHPVC)

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Administrator
U.S. Environmental Protection Agency
Ariel Rios Building
Room 3000, #1101-A
1200 Pennsylvania Avenue N.W.
Washington, D.C. 20460

Dear Administrator:

On behalf of the Flavor and Fragrance High Production Volume Consortia, I wish to thank the Environmental Protection Agency (EPA) for their comments on the test plan and robust summaries on the Chemical Category "Terpenoid Aliphatic Primary Alcohols and Related Substances. The Terpene Consortium, as a member of FFHPVC, serves as an industry consortium to coordinate testing activities for terpenoid substances under the Chemical Right-to-Know Program. Since 1999, the twenty-one (21) companies that are current members of The Terpene Consortium have supported the collection and review of available test data, development of test plans and robust summaries for each of the sponsored chemicals, and conducted additional testing.

Based on our initial recommendations for testing and the peer-reviewed comments of the EPA, the Terpene Consortium of the Flavor and Fragrance High Production Volume Consortia (FFHPVC) is pleased to submit the following revised test plan and robust summaries for the Chemical Category, "Terpenoid Primary Alcohols and Related Esters". The revised test plan and robust summaries contain the results of additional toxicity studies and additional metabolic information that addresses the questions and comments made by the EPA in its letter dated 2/2001. This letter contains responses to the comments made by the EPA. These responses taken together with the inclusion of new study data and other information constitute the key changes to the original test plan and robust summaries. Based on this additional data, we conclude that the current test plan and robust summaries for this chemical category are now complete.

Based on this additional information, the Terpene Consortium concludes that the experimental and model data for physiochemical properties, environmental fate, ecotoxicity, and human health endpoints are consistent for the members of this chemical category. The database of information on category members permits one to reliably predict endpoint values for other untested members of the category. Therefore, these data support the inclusion of the four listed substances in the chemical category and would allow for other structurally related substances to be included in the chemical category.

In the EPA letter dated 19 October 2001, it was pointed out that:

" It may well be, on the basis of experience gained over years of use, that most of the substances have little compelling evidence suggesting that testing is needed in the context of the HPV Challenge Program. Nonetheless, while this line of reasoning could have been used to support the recommendation not to test the substances in this category, the information was only provided as background; few examples, and no actual data, were cited."

Without prior guidance from EPA, the Terpene Consortium felt responsible to report endpoint data for these substances. Most of these data have already been provided to the US Food and Drug Administration and the World Health Organization during their evaluation of these substances as food additives. The three alcohols and one related ester that constitute the members of this chemical category have been reviewed by the World Health Organization/Food and Agriculture Organization Joint Expert Committee for the Evaluation of Food Additives (WHO/FAO JECFA) for use as flavoring substances in food. As part of its responsibility, JECFA maintains on ongoing program of review of the safety of food additives (WHO Technical Series Nos. 38, 40, 42, 44, 46, and 48). In 1997, geranyl acetate (acetylated myrcene) [WHO Food Additive Series: 40, 1998; see Revised Test Plan] and in 2003, geraniol, dl-citronellol and nerol [JECFA, 2003; see Revised Test Plan] were recognized as safe for use in food. In addition, JECFA has assigned an acceptable daily intake of 0.5 mg/kg bw for use of geranyl acetate (acetylated myrcene) in food.

The substances in this category are also recognized as "Generally Recognized as Safe" (GRAS) for their intended use in food by the United States Food and Drug Administration under the Code of Federal Regulations (CFR 172.515). Under supervision of the Food and Nutrition Board of the Institute of Medicine, National Academy of Sciences, specifications for the commercial use of each of these substances in food are published in the Food Chemical Codex [FFC, 1996; see Revised Test Plan].

Based on the long history of use of these substances both as naturally occurring components of food and as substances intentionally added to food, the hazard assessments performed by the US FDA and WHO/FAO JECFA, and the current regulatory status for the addition of these substances to the food supply, there is no compelling evidence that these substances should be further tested for physiochemical properties and human health endpoints in the EPA Chemical "Right to Know" Program. We do, however, maintain that data on the environmental fate and ecotoxicity are relevant to the HPV Challenge program. In this context, we have sponsored numerous ecotoxicity studies to provide a robust

database on ecotoxicity endpoints. We consider that the test plan and robust summaries for this category are final and have no plans to provide additional data. Your comprehensive comments provided the necessary guidance to complete the test plan for this category. The collaboration between the Terpene Consortium and the Environmental Protection Agency in the Chemical “Right to Know” Program has produced a hazard database that will be useful to the public for decades to come. Thank you for the opportunity to participate in such a program.

If you have any questions or comments concerning the contents of this letter, please feel free to contact me at any time (202-331-2325) or tadams@therobertsgroup.net.

Best regards,

Timothy B. Adams, Ph.D.

Technical Contact Person for FFHPVC

FFHPVC Responses to EPA COMMENTS

FFHPVC notes EPA remarks and provides their responses immediately below each comment.

1. Category Justification: In general, the category approach was adequate for the SIDS health endpoints; the sponsor needs to consider whether, for environmental effects, the acetates may not belong in a category with the alcohols (see Test Plan, below).

Environmental and ecotoxicity data have been obtained to fully characterize the environmental effects of the acetate ester in the category (see below, test plan, and robust summaries).

2. Physicochemical and Environmental Fate Endpoints: Adequate data exist for these endpoints for the purposes of the HPV Challenge Program. However, the submitter needs to provide the input values employed in its EPIWIN calculations; provide a more detailed reference when referring to the reported values by FMA and provide more information on methods; and clarify the intended use of the robust summary data for the non-category member citral (CAS # 5392-40-5) (see Test Plan, below).

Input values have been included in the robust summaries for the most recent version of EPIWIN model calculations. Wherever data is available, more detailed information on methods, etc. is provided. Robust summaries for citral (a mixture of neral and geranial) have been deleted.

3. Health Effects: Data are adequate for the purposes of the U.S. HPV Challenge Program. However, some robust summaries are deficient and need to be enhanced (see Specific Comments on Robust Summaries).

Wherever available, additional data has been provided. The sponsor has also added the results of the recent 2-year bioassay performed with citral (the principal metabolite of geraniol and nerol) by the National Toxicology Program (NTP, 2003; see Revised Test Plan).

4. Environmental Effects: EPA agrees with the sponsor's proposal to test geraniol in fish and daphnia. In addition, geraniol should be tested in algae as well because the provided algal test results are inadequate (see detailed comments). EPA disagrees that testing of acetate esters is not necessary, and suggests that geranyl acetate be tested in fish, daphnia, and green algae (see Test Plan, below).

The sponsor has tested geraniol and citronellol for toxicity to fish, aquatic invertebrates, and aquatic plants. Geranyl acetate has been tested for toxicity to fish and an isomeric terpene ester linalyl acetate has been tested for toxicity to aquatic invertebrates and aquatic plants. The results of these tests support the conclusion that terpenoid alcohols and related terpenoid acetate ester exhibit similar ecotoxic potential.

EPA COMMENTS ON TERPENOID PRIMARY ALCOHOLS AND RELATED ESTERS CATEGORY CHALLENGE SUBMISSION

To support inclusion of the acetates as category members for environmental effects, the submitter cites studies of the enzyme carboxylesterase in fish and implies that the primary chemical exposure for fish is thus the hydrolyzed alcohols. EPA believes that this approach is an oversimplification; see the discussion of this point in the Test Plan section below.

Based on the results of acute toxicity testing of the ester and the corresponding alcohols in fish and daphnid, it can be concluded that both substances exhibit similar levels of toxicity. The LC values are on the same order of magnitude. This evidence supports metabolic evidence that the acetate ester, being a naturally occurring terpene persistent in the environment, undergoes partial or complete hydrolysis.

The thrust of the category justification was health endpoint-related. Adequate attention should be given to justify the category approach for the other endpoint areas.

Test Plan

Chemistry (melting point, boiling point, vapor pressure, water solubility, and partition coefficient)

Data for these endpoints are adequate for the purposes of the HPV Challenge Program.

Boiling Point: The boiling points for geranyl and neryl acetate are incorrectly reported in the text of the test plan but are correctly reported in the Robust Summaries.

Water Solubility: In the Test Plan, the submitter reports a water solubility value for citronellol of 600 mg/L, and for geraniol a value of 300 mg/L. However the Robust Summaries report values of 300 mg/L for citronellol and 600 mg/L for geraniol. The submitter needs to reconcile these disparities.

The robust summaries and test plan have been corrected to accurately reflect the data reported in the test plan and robust summaries.

Environmental Fate (Photodegradation, Stability in Water, Biodegradation, Fugacity)

Data for these endpoints are adequate for the purposes of the HPV Challenge Program.

The submitter provides photodegradation and biodegradation robust summaries for Citral (CAS # 5392-40-5), a mixture of geranial and neral. The test plan cites only the biodegradation results, without explanation. The reason for including the material should be explicitly stated.

Since neral and geranial (citral) are the intermediary oxidation metabolites of nerol and geraniol, data on the biodegradation of citral demonstrates that even the oxidation metabolites of the respective alcohols are readily biodegradable. Although not required for the endpoint, these data provide additional insight into biodegradability of the alcohol and their reaction products.

Chemical Transport and Distribution in the Environment: The submitter indicates in its Test Plan that it estimated the transport and fugacity of these chemicals using the Level I Fugacity-based Environmental Equilibrium Partitioning Model. EPA recommends using the EQC Level III model; see Specific Comments on the Robust Summaries. Because some of these chemicals have experimental biodegradability and other data available, in those cases the sponsor should run the Level III fugacity model using the appropriate test results.

The fugacity model predictions have been recalculated using the EQC level III Model.

Health Effects (acute toxicity, repeat dose toxicity, genetic toxicity, and reproductive/developmental toxicity).

EPA agrees with the overall test plan not to conduct further SIDS testing and has the following comments:

Reproductive Toxicity. The "two-generation" reproductive toxicity study in rats appears to be a one-generation study with an unusual protocol in that only females were exposed to the test material. The fact that male reproductive organs were evaluated in several repeat dose studies with other category members allows this SIDS endpoint to be met for the purposes of the U.S. HPV Challenge Program. In addition, EPA agrees with the submitter's argument that citral (a mixture of geranial and neral) is representative of the category because of the alcohol-to-aldehyde conversion likely to occur in mammals.

Developmental Toxicity. As stated above for reproductive toxicity, EPA agrees with the argument for using the citral data (in this case, appropriately run studies were conducted) to represent the category.

Environmental Effects (Fish, daphnia and algae toxicity)

Geraniol. EPA agrees with the sponsor's proposal to test geraniol in fish and daphnia.

Geranyl acetate. As the test plan states, under environmental conditions the ester hydrolysis half-life is over 23 days, indicating the likelihood of initial aquatic exposure to the parent esters. However, the sponsor avers that testing of esters is unnecessary because of metabolic considerations, arguing (Test Plan, Section 2.5) that the enzyme carboxylesterase exists in fish and therefore the hydrolysis of primary terpenoid acetates to the corresponding alcohols occurs readily in fish. The test plan thus implies that the primary chemical exposure for fish is the corresponding alcohol. This contention is particularly important because esters generally have higher aquatic toxicity than predicted by the baseline narcosis model (ECOSAR, MS-Windows Version 0.99f, USEPA; Lipnick, RL, "A QSAR study of Overton's Data on the Narcosis and Toxicity of Organic Compounds to

the Tadpole, *Rana tempraria*." In: ***Aquatic Toxicology and hazard Assessment***: 11th Symposium, GW Suter, II and M Lewis, eds., American Society for Testing and Materials, STP 1007 Philadelphia, PA, 1989, pp. 468-489). EPA believes that the postulated metabolic hydrolysis to the alcohol does not preclude aquatic exposure to the ester. Moreover, it is not known if this enzyme is present in invertebrates, and it is likely not present in algae. Therefore, in order to fully characterize the aquatic toxicity of this category, EPA suggests that geranyl acetate be tested in fish, daphnia, and green algae. Given the preceding discussion, the sponsor needs to consider whether, for environmental effects, including the esters in this category may not be appropriate.

All aquatic testing should follow the Guidance Document on Aquatic Toxicity Testing of Difficult Substances and Mixtures (OECD, June 2000-available on the OECD website at http://www.oecd.org./ehs/test/monos.htm).

Ecotoxicity studies have been performed for geraniol, citronellol, geranyl acetate and linalyl acetate in fish daphnid, and algae using standardized OECD protocols.

A number of ECOSAR estimations were included in the robust summaries. These robust summaries were inadequate due to insufficient detail on the data inputs. The sponsor used several measured log P values to predict toxicity instead of calculated log P values as the model used to develop the SAR regression equations. The use of SAR in this manner may give varying results, as illustrated by comparing the predicted and measured aquatic toxicity values submitted for certain chemicals. The sponsor needs to justify using measured log P as an input into the ECOSAR model. The sponsor noted the excess predicted toxicity of geraniol and nerol was due to ECOSAR's treatment of allyl alcohols geraniol and nerol as vinyl alcohols rather than neutral organics. EPA notes that this issue should be adequately addressed by the test results on geraniol.

The sponsor agrees with the EPA. The ecotoxicity values have been recalculated using model log P data. Upon completion of the ecotoxicity tests for geraniol, it can be concluded that the model prediction for geraniol and nerol based on their classifications as vinyl alcohols produces conservative estimates for ecotoxic endpoints. The model would be best served if these alcohols would be reclassified as alcohols.

Fish. EPA agrees with the sponsor's plan to test geraniol using OECD guideline 203 to determine the 96-hour fish LC_{50} and then use the read-across approach for nerol and dl-citronellol. However, EPA also suggests testing of geranyl acetate owing to potential ester toxicity as outlined above. The fish test should be conducted using a closed system with no head space and mean measured concentrations.

Daphnia. EPA agrees with the sponsor's plan to conduct a test on geraniol using OECD guideline 202. EPA also suggests that the sponsor consider testing geranyl acetate for daphnia owing to the ester toxicity rationale already mentioned. The daphnia test should be conducted using a closed system with no head space and mean measured concentrations.

Algae. EPA suggests that the sponsor consider testing geraniol because of inadequacy of reported data and test method. Available studies used an unacceptable test method for green algae (see Specific Comments on the Robust Summaries), yielding results that are qualitative and difficult to associate with the quantitative effects that would be anticipated in the environment. EPA also suggests that the sponsor consider testing geranyl acetate in algae owing to the ester toxicity rationale already mentioned.

The sponsor agrees with the EPA assessment for ecotoxicity endpoints. Studies for each endpoint have been performed for geraniol and citronellol. Studies on geranyl acetate and linalyl acetate (a terpene isomer) have also been performed for the three ecotoxicity endpoints. The results are mutually consistent and confirm the category membership.

Specific Comments on the Robust Summaries

Physicochemical Properties

The boiling point and vapor pressure robust summaries cite "Fragrance Materials Association (FMA) reported values." Furthermore, it is not clear whether the boiling point data provided are measured or calculated. The submitter should provide a more detailed reference for the FMA data and provide method/guideline information.

Whatever detail was available was included in the revision. The boiling points are measured FMA values

Water Stability. The submitter cited AOPWIN (which estimates photodegradation) rather than HYDROWIN as the method for estimating the hydrolysis of acetylated myrcene.

The reference was incorrectly given. The reference was corrected.

Fate

The submitter should provide input values to its Environmental Fate calculations so the data can be adequately evaluated.

All transport and distribution results were estimated using the Level I Fugacity-based Environmental Equilibrium Partitioning Model. EPA recommends the EQC Level III model, which is more realistic and useful for estimating a chemical's fate in the environment, using experimentally determined values such as biodegradation half-life where available.

In order to develop the Level III fugacity model, EPA recommends using the EQC Level III model from the Canadian Environment Modeling Centre at Trent University, which allows full control of data inputs. This model can be found at the following web address: http://www.trentu.ca/academic/aminss/envmodel.

Health Effects

Many of the health endpoint summaries were not considered adequate or were deficient (i.e., need to be enhanced) for the purposes of the U.S. HPV Challenge Program. That some robust summaries are considered inadequate does not mean that new testing is necessary, however, because other existing data with adequate summaries are presented. Specific comments follow:

Acute Toxicity

The respiratory irritation studies in female mice with citronellol, geraniol, and nerol are inadequate because they are not appropriate protocols to assess acute toxicity/lethality. The tests were designed to assess respiratory irritation following exposure periods of one minute.

The reliability code for these summaries have been changed to Klimisch Code No. 3, inadequate.

Genetic Toxicity

Chromosome aberration test in Chinese hamster fibroblast cells with geraniol (Ishidate et al., 1984): There is no rationale for choosing the maximum concentration tested. In addition, there is no rationale presented for not conducting the experiment under metabolic activation conditions. This information is necessary to assess the validity of the test.

The information was not available in the article. The reliability code has been changed.

Ames test with acetylated myrcene (Mortelmans et al., 1986): Only 70% of the test substance was identified; the identity of the remainder should be provided.

There was no information for the identify of the secondary components in the published article.

Repeat Dose Toxicity

Twelve week study with citronellol and linalool mixture (Oser, 1958): This robust summary is inadequate because there is no documentation of the relevance of the study design to systemic toxicity. The study was conducted primarily to assess the effects of the test substances on food utilization and the primary endpoints assessed were urinary output of sugar and albumin.

The reliability code for this study has been changed to reflect EPA comments. The study is what historically was recognized as a safety evaluation study. It contained basic elements of toxicologic investigation including measurement of body and organ weights (liver and kidneys), gross and histopathological examination, and clinical chemistry and hematology measurements.

Reproductive Toxicity

"Two-generation" study of citral in rats (Hoberman et al., 1989): As noted above, this study appears to be a one-generation study in which only females were given the test substance. This robust summary is deficient in that the incidence by dose for all effects noted needs to be provided.

Additional detail is provided, if available.

Developmental Toxicity

Reproductive/developmental toxicity screening study with geraniol in rats (Vollmuth et al., 1990): This robust summary is deficient because: (1) the test substance was not adequately characterized, and (2) the incidence by dose for all effects noted needs to be provided.

Additional detail is provided, if available.

Two citral developmental toxicity studies (Cristina et al., 1995; Gaworski et al., 1992): In both cases, the robust summaries are deficient because the incidence by dose for all effects noted need to be provided.

Additional detail is provided, if available.

Environmental Effects

ECOSAR estimates. The sponsor suggests that the ECOSAR estimates for the 96-hour fish LC_{50} values for all substances except dl-citronellol were "overly conservative," while the remainder of the ECOSAR estimates were considered "reliable." The rationale used by the sponsor to determine that the fish toxicity estimates are "overly conservative" is based on one LC_{50} test in sunfish using limonene (an unsaturated cyclic hydrocarbon). No elaboration of the basis for this conclusion was provided in either the Test Plan or the robust summaries.

There was no intention to use the data on limonene to support the conclusion that the LC50 values of the alcohols in the category were overly conservative. This issue has been resolved by the addition of fish toxicity data from at least 6 separate studies.

Algal toxicity. The sponsor reported the results of a set of studies where increasing concentrations of citronellol, geraniol, nerol, and citral were applied to disks placed on agar plates seeded with the alga *Chlorella p.,* then positioned under fluorescent lights for 48 hours. The plates were then examined for inhibitory effects. Interpreting the results of these growth inhibition tests is difficult due to limitations in quantifying the results. In addition, the algorithms used by ECOSAR to estimate algal toxicity were not developed using the inhibition test reported in the Test Plan. Therefore, an analogy cannot be made and geraniol and its acetate should be tested using standard guidelines such as OECD guideline 201. The algal test should be conducted using a closed system with no head space and mean measured concentrations.

The algae test for geraniol, citronellol, and linalyl acetate by OECD 201 Guidelines provide consistent data for conclusions concerning algal toxicity.